

Figure 1 General principle of chemical ligation.

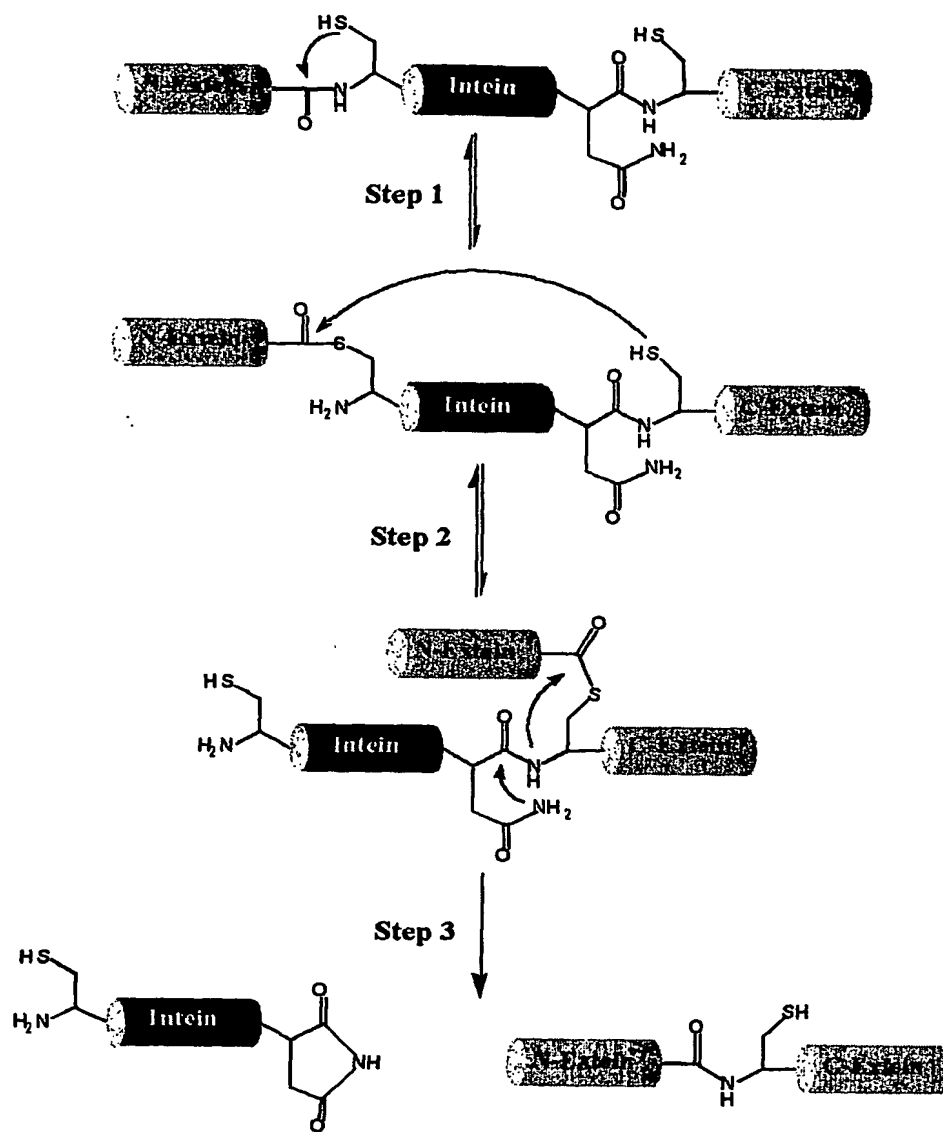


Figure 2 Mechanism of protein splicing

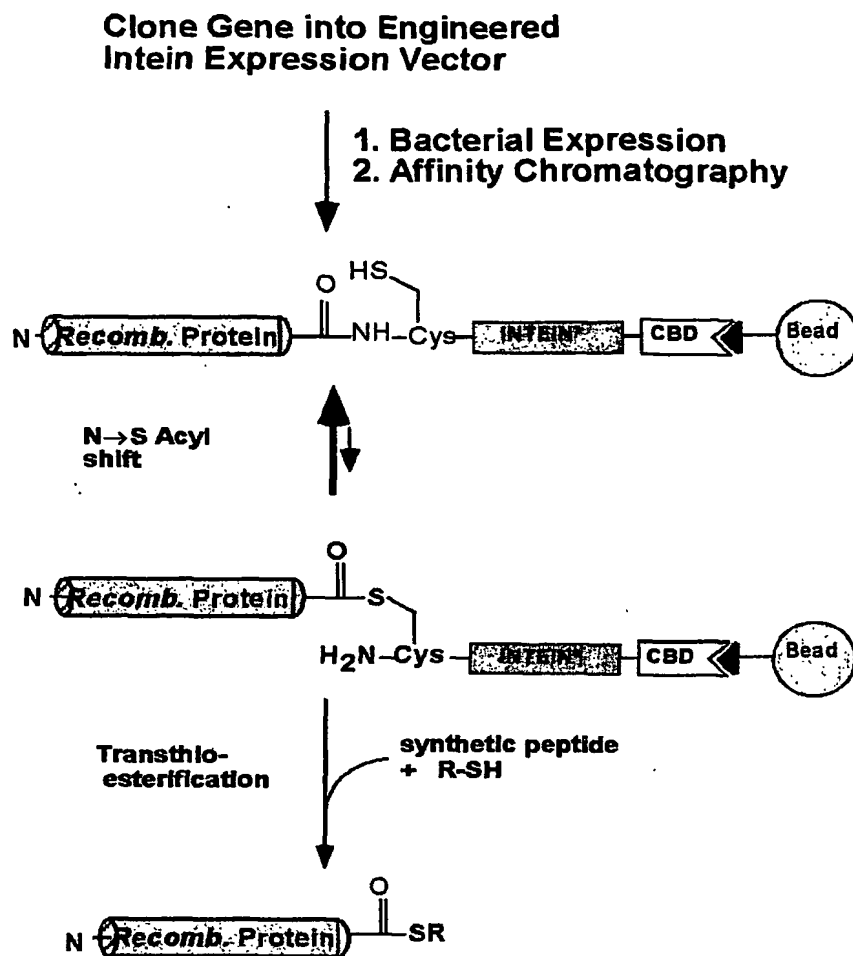
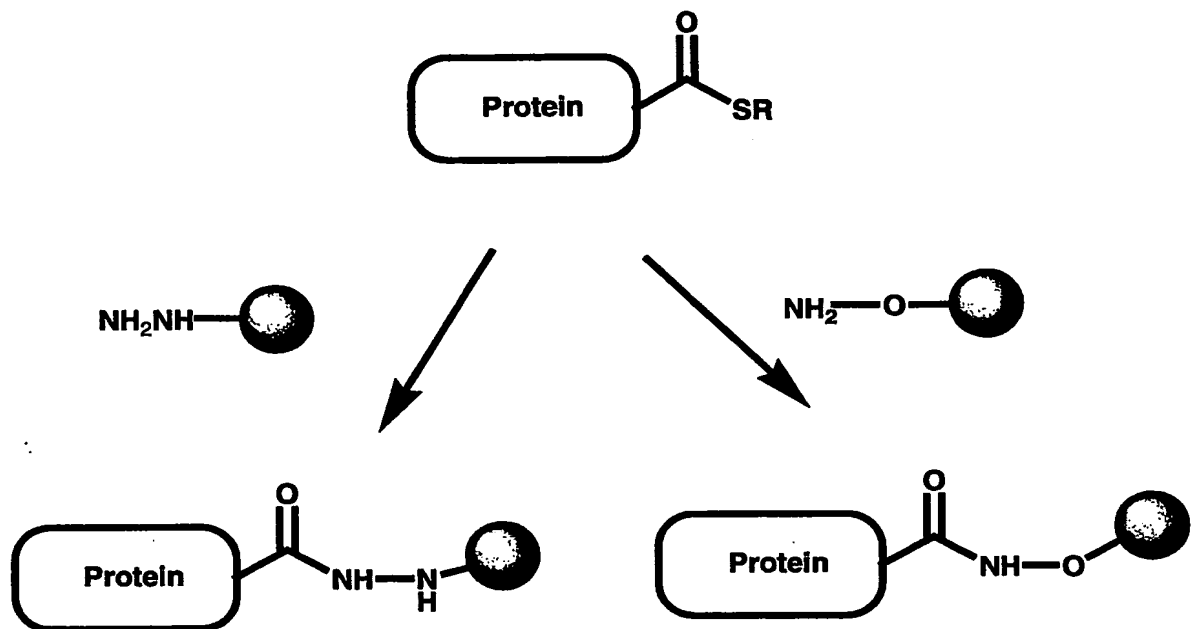


Figure 3 Generation of Recombinant C-terminal Thioester Proteins

**Synthetic or recombinant peptide / protein  $\alpha$ -thioester**

**Figure 4** Ligation of protein and peptide thioesters with hydrazine and aminoxy containing entities such as labels, peptides and proteins.

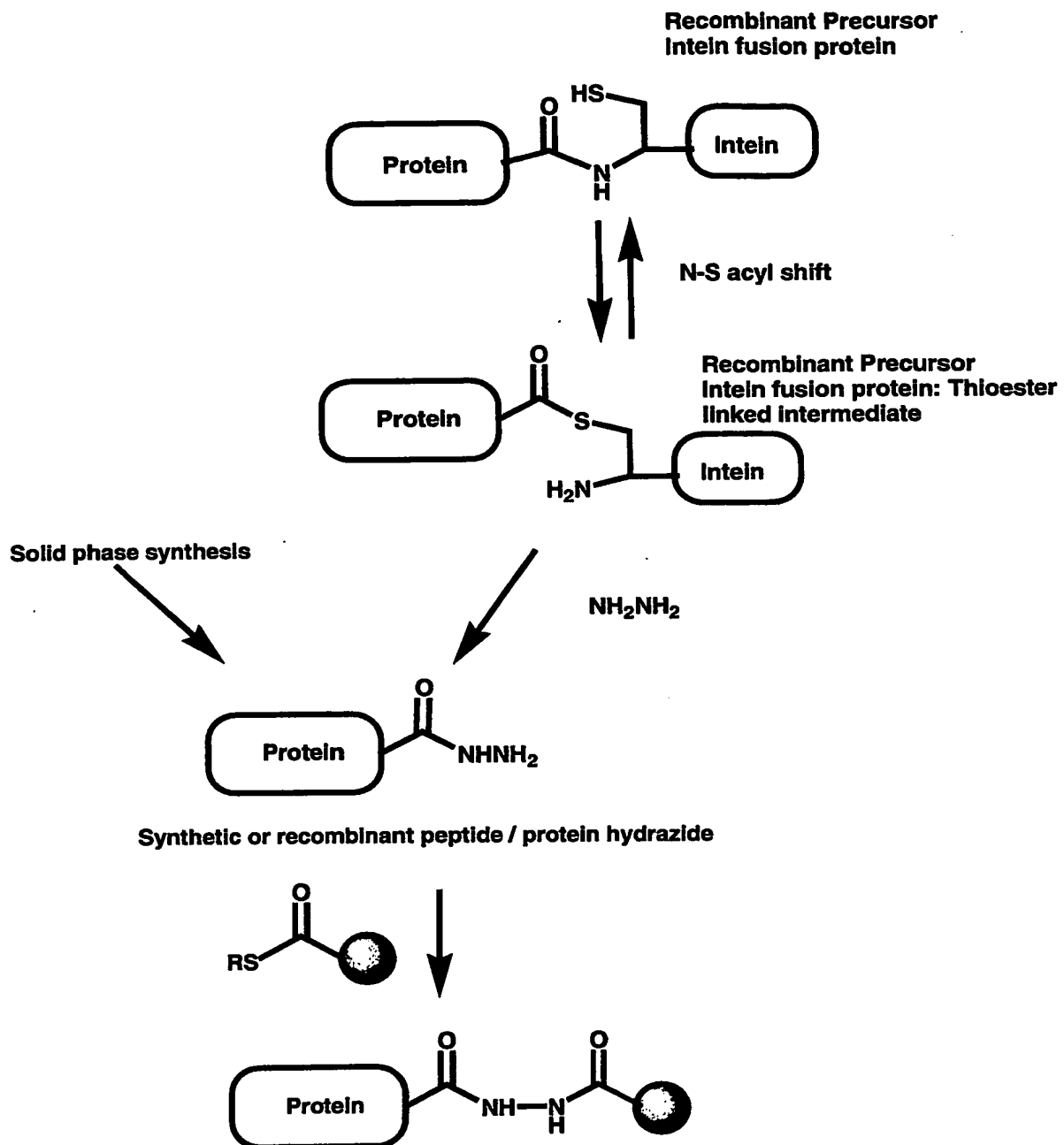


Figure 5 Generation of synthetic and recombinant peptide hydrazides for ligation with thioester containing molecules

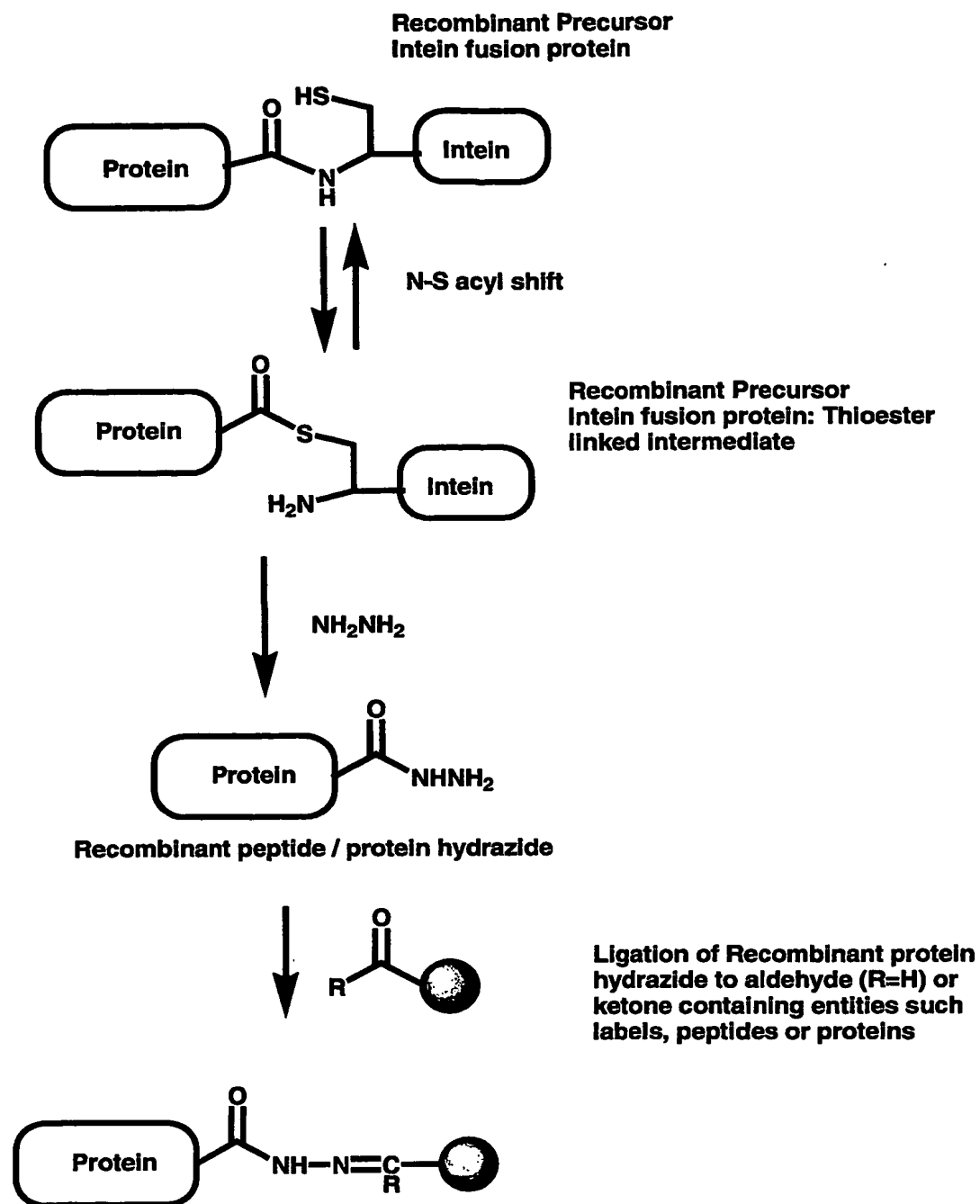


Figure 6 Generation of recombinant peptide hydrazides for ligation with aldehyde and ketone containing molecules

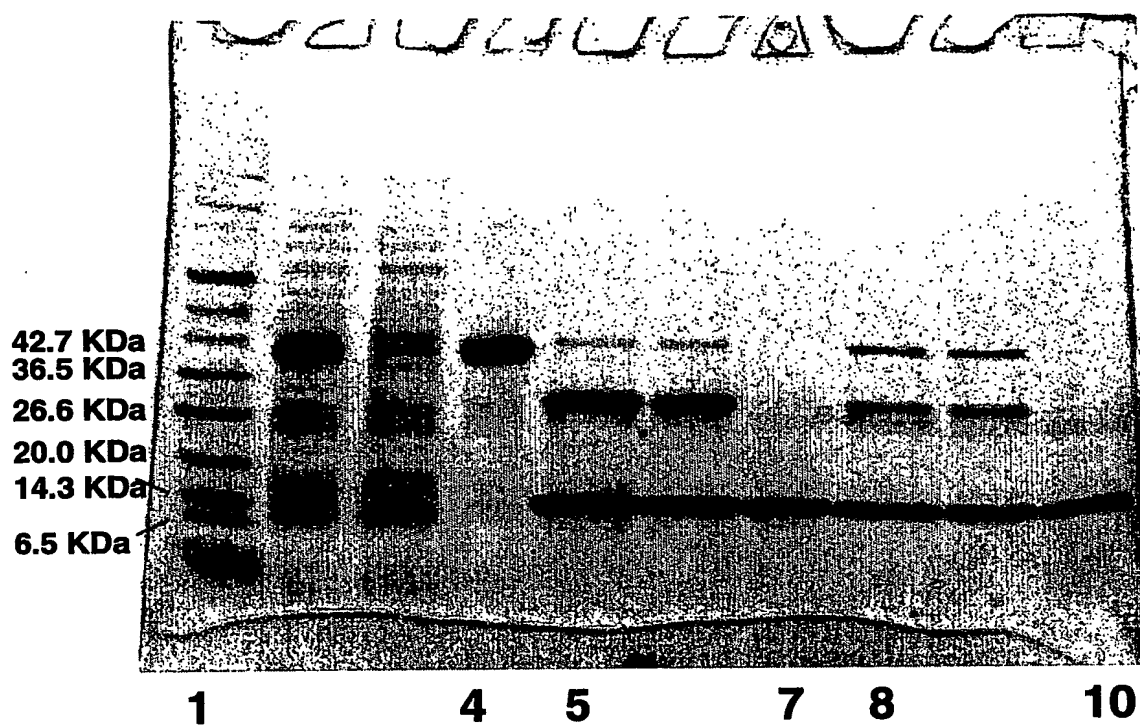


Figure 7

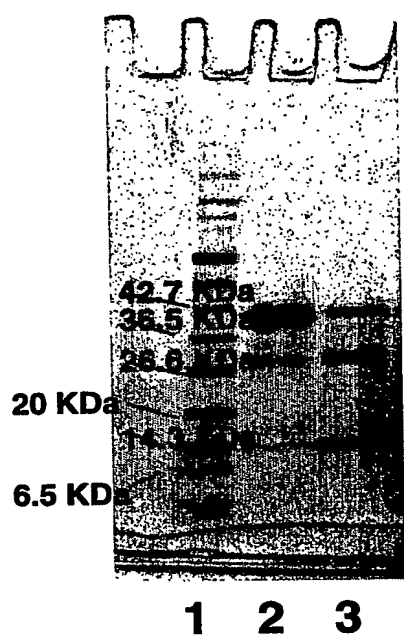


Figure 8



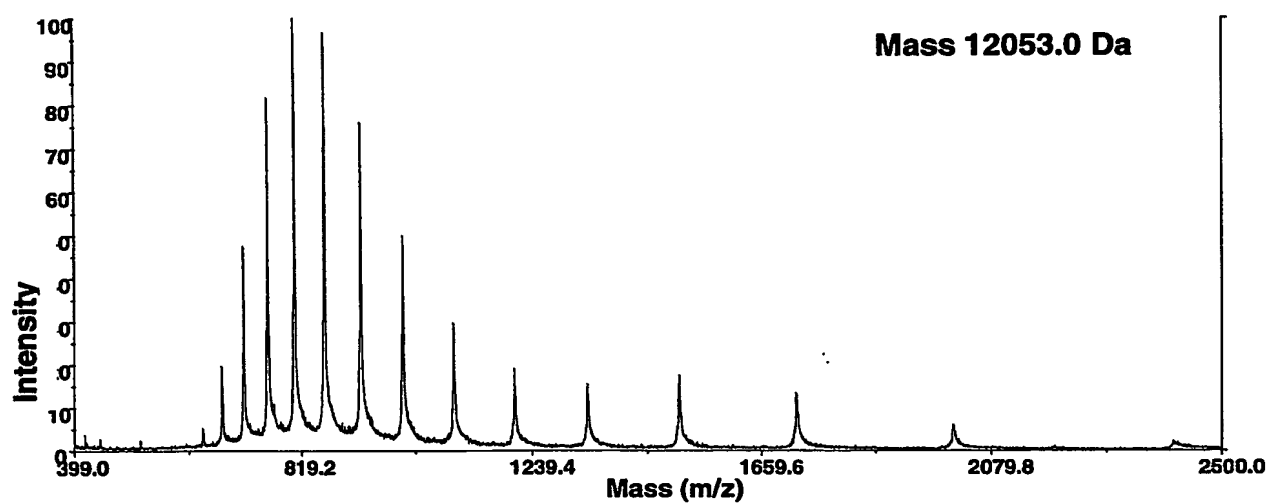


Figure 9. ESMS spectrum of the C-terminal hydrazide derivative of Grb2-SH2

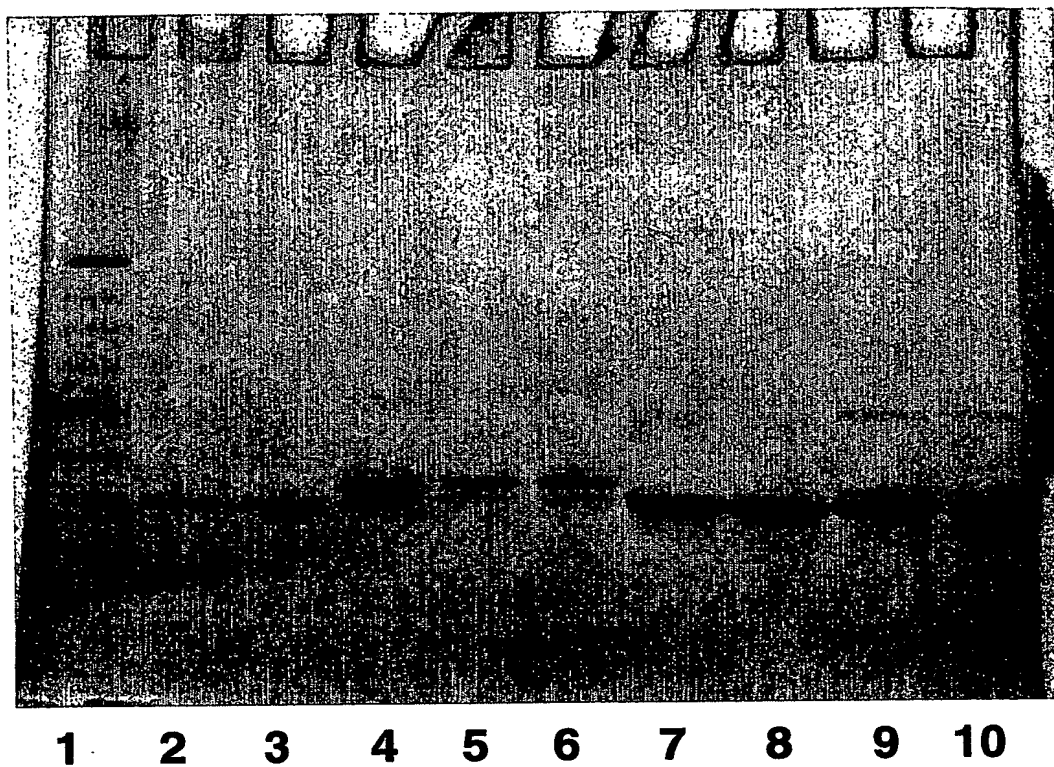


Figure 10. SDS-PAGE analysis of the reaction between synthetic ketone containing peptide CH<sub>3</sub>COCO-myc with Grb2-SH2 – C-terminal hydrazide and Cytochrome C.

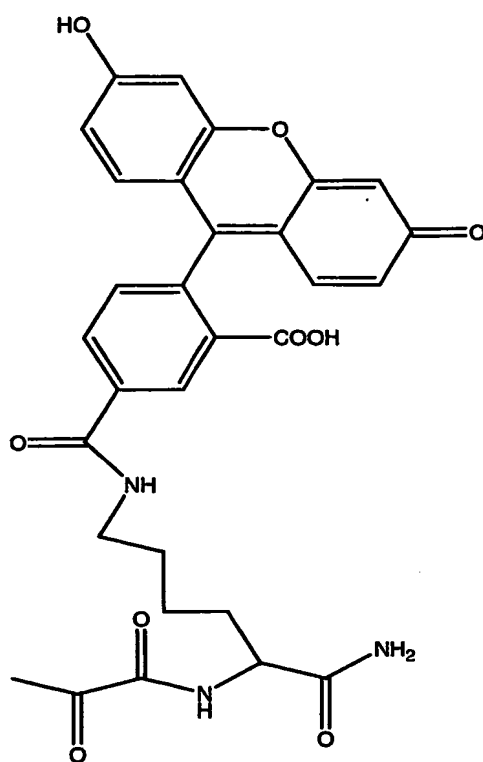


Figure 11 Structure of  $\text{CH}_3\text{COCO-Lys(Fl)}$ .

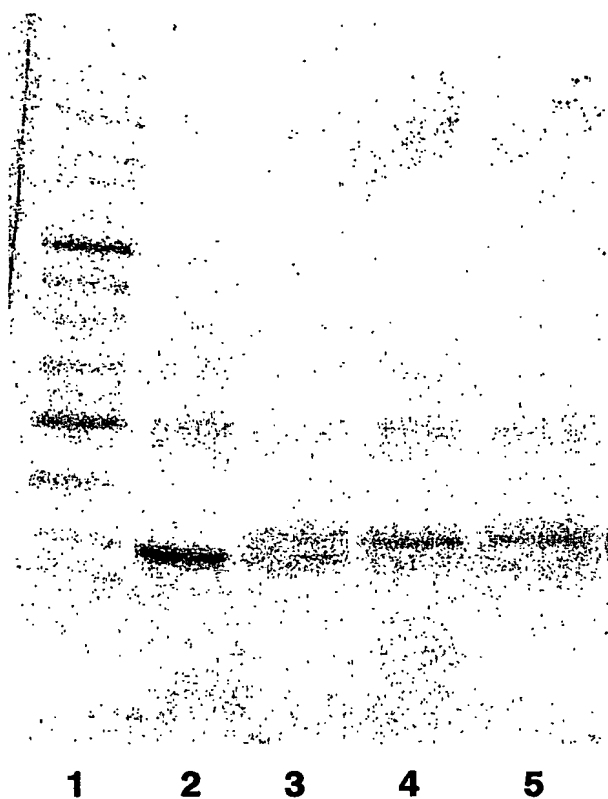


Figure 12

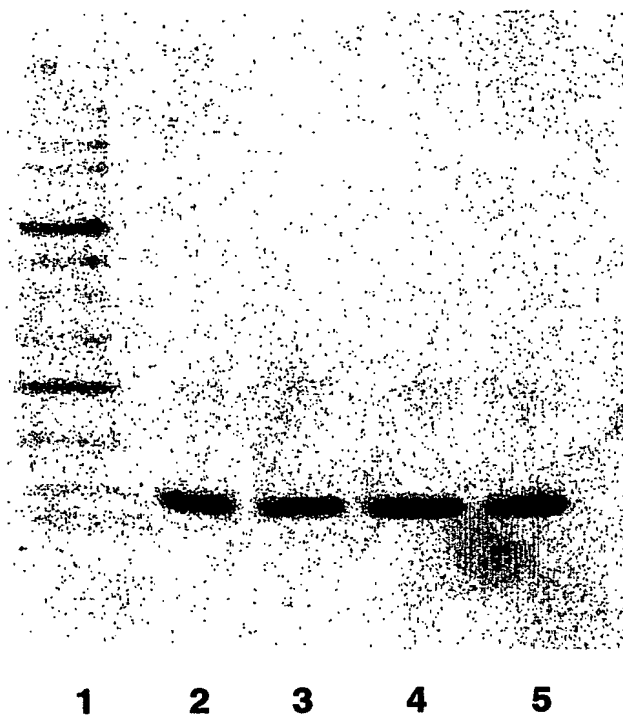


Figure 13

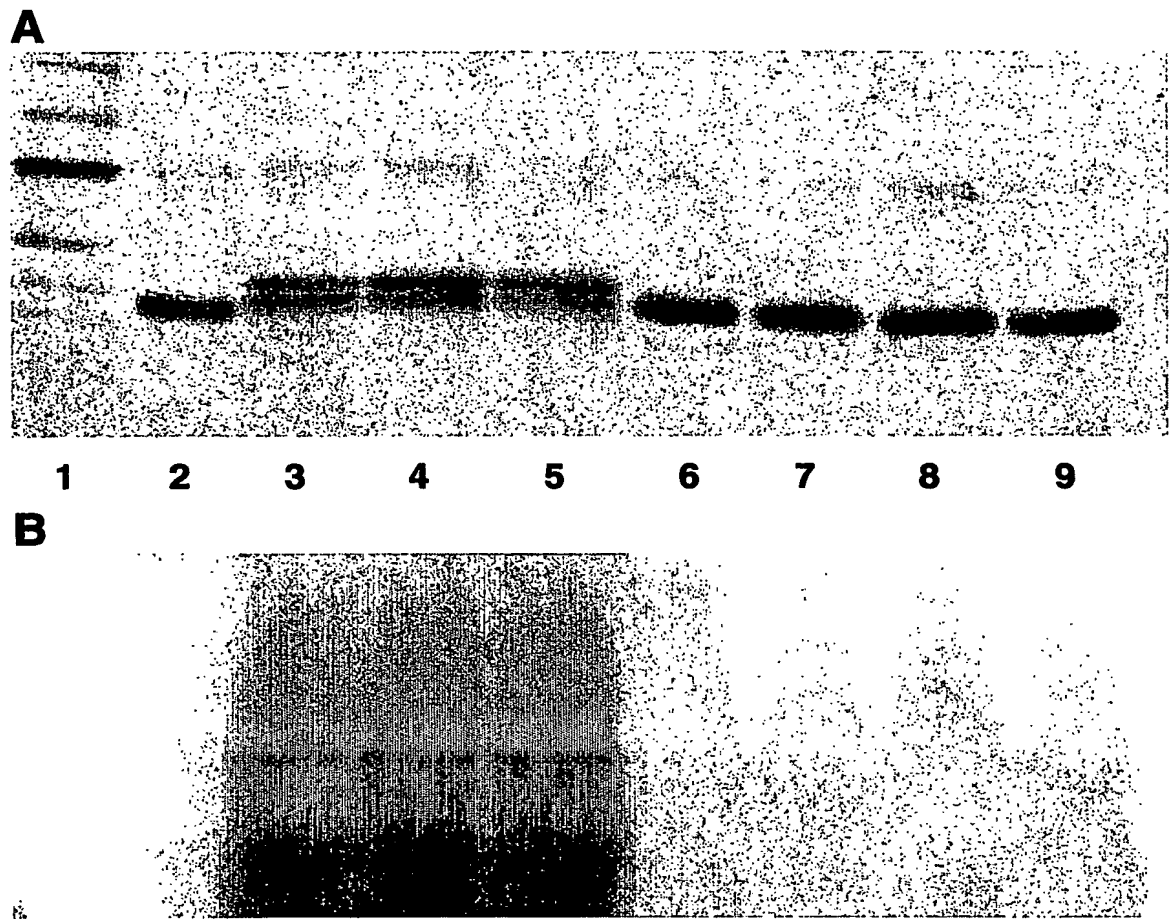


Figure 14

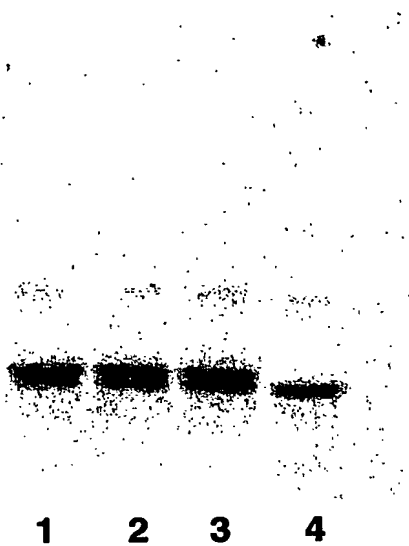


Figure 15.